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New Approaches to HIV Protease Inhibitor Drug Design II: Testing the substrate envelope hypothesis to avoid drug resistance and discover robust inhibitors

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Abstract

Purpose of review—Drug resistance occurs as a result when the balance between the binding of inhibitors and the turnover of substrates is perturbed in favor of the substrates. Resistance is quite wide spread to the HIV-1 protease inhibitors permitting the protease to process its ten different substrates. This processing of the substrates permits the HIV-1 virus to mature and become infectious. Designing HIV-1 protease inhibitors that closely fit within the substrate binding region is proposed to be a strategy to avoid drug resistance.

Recent findings—Co-crystal structures of HIV-1 protease with its substrates define an overlapping substrate binding region, or substrate envelope. Novel HIV-1 protease inhibitors that were designed to fit within this substrate envelope, were found to retain high binding affinity and have a flat binding profile against a panel of drug resistant HIV-1 proteases.

Summary—Avoiding drug resistance needs to be considered in the initial design of inhibitors to quickly evolving targets such as HIV-1 protease. Using a detailed knowledge of substrate binding appears to be a promising strategy for achieving this goal to obtain robust HIV-1 protease inhibitors.

Keywords

Drug Resistance; HIV-1 Protease; Substrate envelope; Structure based drug design

Introduction

As the worldwide AIDS pandemic continues, a cure for HIV-1 still eludes the medical community [1]. Although many patients have had complete response to HAART [2,3], reports of failure, partial response, and/or breakthrough with antiretroviral treatment, as measured by viral load, however, have compromised the future of HIV-1 treatment [4,5]. Viral resistance has been recognized as one of the most important factors involved in therapeutic failure [6,7]. A comprehensive understanding of the development of HIV-1 resistance to antiretroviral agents is critical to improving therapeutic management [8–11]. Protease inhibitors are essential components of most HAART therapies [12,13].

The effects of mutations in HIV protease as in all the HIV proteins is a constant issue in inhibitor design as the HIV-1 reverse transcriptase is inherently inaccurate. Mistranslation of 1 in every 10,000 codons [14] results in a very high rate of mutation in all the viral proteins [15,16]. Since

the introduction of protease inhibitors, drug-resistant mutations in the protease have become widespread. Once a primary drug resistant mutation occurs, other secondary mutations often occur which increase the fitness of the protease. Therefore, not only must an inhibitor, or cocktail of inhibitors, recognize and bind tightly to one protein but the protease inhibitor must effectively target a whole family of closely related enzymes.

Protease Inhibitors and Drug Resistance

HIV protease inhibitors were the first success of structure-based drug design [17]. Currently there are nine FDA-approved HIV-1 protease inhibitors, indinavir (IDV), nelfinavir (NFV), amprenavir (APV), saquinavir (SQV), ritonavir (RTV), lopinavir (LPV), atazanavir (ATV), Tipranavir (TPV) and Darunavir (DRV), all of which are competitive inhibitors binding at the active site. Most of the inhibitors, even those whose precursors were found through screening libraries, were optimized with successive co-crystal crystal structures [18–24]. These drugs, often the first lines of treatment for infected patients as they are well tolerated, are peptidomimetics that resulted from structure-based drug design efforts of both academia and the pharmaceutical industry. All of them have large, generally hydrophobic, moieties that interact with the mainly hydrophobic P2-P2' pockets in the active site [17] and all but tipranavir [23] are peptidomimetics. Although the currently prescribed HIV-1 protease inhibitors are all chemically different [25,26], relatively low molecular weight compounds, the three-dimensional shape and electrostatic character of these drugs are fairly similar. These inhibitors can elicit different, yet overlapping, patterns of drug resistant mutations [27–29], therefore a relatively small set of mutations can result in a protease variant with multi-drug resistance.

In fact mutations in at least 34 of the 99 residues of HIV-1 protease have been found to have clinical significance [29–34]. Only a subset of these mutations, such as D30N, G48V, V82A, I84V, I50V, and I50L, affect inhibitor binding by an alteration of a direct point of contact within the active site. Certain of these predominantly are closely associated with a particular inhibitor such as D30N with NFV, G48V with SQV, I50V with APV and DRV or I50L with ATV, others such as V82A and I84V impact almost all of the inhibitors. Many other mutations alter inhibitor binding by altering the balance between substrate recognition and inhibitor binding. HIV-1 found in most highly experienced patients has between 5 and 15 mutations in the protease gene [29,33,35,36]. These are often in specific combinations of mutations both inside and outside the active site. Some common sites outside the active sites are L10I, I54V or T, A71V or T, V77I, and L90M. Mutations outside the active site may not only impact inhibitor binding but also compensate for the viability and fitness of the enzyme and thus increase the growth rate of the mutant virus. The commonality of many of these mutations potentially limits the success of subsequent therapy presenting a new challenge to future structure-based drug design efforts.

Substrate Recognition and Drug Resistance

In general drug resistance occurs when mutations in the target protein enable it to retain function while no longer being effectively inhibited by the drug [37]. For HIV-1 protease these mutations render the variant protease resistant to the inhibitor while allowing it to maintain its function in cleaving its ten natural substrates [37–39] in the Gag and Gag-Pro-Pol polypeptides. By exploring how HIV-1 protease recognizes its diverse non-homologous substrates [39] in atomic detail, insights have been gained into one possible means for circumventing drug resistance. The co-crystal structures of HIV-1 protease with a series of its substrates define an overlapping substrate binding region, or “substrate envelope”, where the majority of the substrates bind [39]. This region or shape is asymmetric and likely defines the recognition motif by which HIV-1 protease recognizes particular sequences as substrates.

Although the inhibitors are much smaller than the substrates, to maintain bioavailability, and are on average a different shape than the substrates. Similar functional groups within the inhibitors are often positioned at similar locations in the protease active site. This overlap means that many inhibitors contact the protease at the same residues [37–39]. Overlaying the inhibitors on the substrate envelope results in several locations, specifically between the P3 and P2' subsites, where the inhibitors protrude beyond the substrate envelope. In fact, the location of most of the primary active site drug-resistant mutations in HIV protease occur exactly where the inhibitors protrude beyond the substrate envelope [37]. Many of these mutations are associated with multi-drug resistance. The extent to which a given inhibitor fits within the envelope can be used to predict the extent to which inhibitors will likely be susceptible to resistance [40,41]. Reinforcing the conclusion that drug resistance occurs at residues that are more important for inhibitor binding than for substrate recognition, as mutation of them adversely impacts inhibitor binding with minimal effect on substrate processing.

A key implication of this analysis is that an inhibitor contained within the substrate envelope, interacting only with the same residues that are necessary to recognize substrate, may be less susceptible to drug resistance. Development of such an inhibitor should be feasible as the picomolar inhibitor DRV, although not designed with this constraint, fits reasonably well within the substrate envelope [37,42] and resistance only occurs in the clinic with many simultaneous mutations. Therefore, developing inhibitors to fit within the substrate envelope represents a new paradigm for drug design that should be less susceptible to drug resistance.

Designing inhibitors using the substrate envelope hypothesis

In a recent series of studies novel inhibitors were designed to test the substrate envelope hypothesis [43–46]. These inhibitors were designed based on (*R*)-(hydroxyethylene) sulfonamide isostere, similar to APV and DRV. The scaffold was chosen because it fits predominately within the substrate envelope, easy to synthesize and has three sites for functional group variability. Inhibitors were designed and synthesized by varying three substituent groups on the (*R*)-(hydroxyethylene)sulfonamide isostere and tested against a panel of drug resistant forms of HIV-1 protease to evaluate the substrate envelope hypothesis. Three design methods were used to develop inhibitors, a more traditional structure activity (SAR) synthetic method and two computational that explicitly incorporated the substrate envelope hypothesis. The two computational methods utilized optimized docking and inverse design libraries respectively. The resulting inhibitors from the three design efforts had affinities that ranged from micromolar affinity to picomolar.

The SAR library [46] was designed and synthesized based on the (*R*)-(hydroxyethylene) sulfonamide isostere scaffold without using any explicit substrate envelope constraint. A heterocyclic moiety with multiple polar atoms, N-phenyloxazolidinone-5-carboxamide, was used to replace the interactions made by Tetrahydrofuran moieties in APV/DRV. Substituents were varied on the phenyl group at the nitrogen of the oxazolidinone ring. The binding affinities of the SAR library inhibitors were in the range of 250 nM to 0.8 pM, however, against a panel of drug resistant variants the highest affinity of these inhibitors lost 100–1000 fold in binding affinity.

Optimized docking created a combinatorial library extensively varying the three positions [45]. The resulting compounds were evaluated using a combination of the energetic favorability of docking and how close within the substrate envelope the inhibitor fit. Finally, a genetic algorithm predicted the optimal combinations of the most promising substituents. This led to the synthesis of a small library of compounds, the tightest of which bound with 24 nM affinity. Although relatively weak, the tightest of these inhibitors had a fairly flat binding profile against

a panel of resistant variant, relative to NFV, LPV, IDV, SQV and RTV. Co-crystal structures revealed that, as designed, these inhibitors fit within the substrate envelope.

The inverse design library [43] searched over a discrete space of substituent groups to identify molecules that did not extend outside the substrate envelope and were complementary to the HIV protease active site. Two rounds of design and synthesis were performed. The first set resulted in inhibitors with binding affinities from 26 μ M to 30 nM, and a flat binding profile, with the tightest inhibitors not losing more than 15-fold in affinity to a panel of resistant variants. A second re-optimized library designed on a high affinity inhibitor-protease complex (1T3R) [42] instead of substrate-protease complex (1KJG) [39] resulted in inhibitors with binding affinities from 4.2 nM to 14 pM (or 1000-fold higher than in the first library). A subset of those inhibitors retained robust flat subnanomolar binding affinity to a panel of resistant variants and their co-crystal structures once again demonstrated that they stayed closely within the volume of the substrate envelope. Suggesting that designing inhibitors using the substrate envelope may be a useful strategy in the developing of HIV-1 protease inhibitors with low susceptibility to resistance.

Additional challenges to inhibitor design from sequence evolution

Development of inhibitors that fit within the substrate envelope will likely decrease the probability of resistance arising. This decrease in the probability of resistance seems likely as a mutation that would alter the inhibitor binding by changing a direct contact of the inhibitor would simultaneously impact the recognition of the substrates. Nevertheless, the impact of mutations outside of the active site also impacts evolution of drug resistance. These include changes in the core of HIV-1 protease either due to natural differences in the HIV clades [47] or compensatory mutations to maintain fitness or can involve changes in substrate sequence. Changes in the beta-barrel core of the protease monomers have been associated with compensatory mutations, such the existence of N88D allowing the simultaneous occurrence of D30N and L90M [48]. Change in the core has also been potentially implicated with the dynamics of the enzyme [49], as HIV protease undergoes a large conformational change to process and release its substrate. The binding of inhibitors to the protease should be less dynamic as the inhibitor should stay tightly bound. Mutations that increase the flexibility of HIV-1 protease may detrimentally impact inhibitor binding by increasing the rate of dissociation between the protease and the inhibitor. Changes in the substrates can also alter the balance between inhibition and substrate processing. Substrates have been observed to vary, sometimes coupled with specific protease mutations, the best characterized is Gag A431V occurs often together with the protease mutations V82A [50,51] making the nucleocapsid-p1 cleavage site a better more accessible substrate. Other substrate cleavage site mutations [52] have been observed and may influence on the level of resistance. Any of these sequence variations within the core of the protease and/or within the substrate can alter and modulate the balance between substrate processing and inhibitor binding, and remains a challenge for future inhibitor design.

Conclusions

Drug resistance occurs when the balance between the binding of inhibitors and substrate processing is perturbed in favor of the substrates. Designing HIV-1 protease inhibitors that closely fit within the substrate envelope is a promising strategy to obtain robust HIV-1 protease inhibitors that are less susceptible to drug resistance. Through such a strategy a mutation that directly impacts the inhibitor binding will also impact the recognition of the majority of the substrates, although the power of viral evolution will likely elicit alternative pathways to resistance. Other strategies [53–57] of restricting inhibitors to predominantly interact only with backbone or conserved side chains to avoid resistance are not necessarily mutually exclusive

with this theory, in fact both cases may be true. Ideally the strategy of using such theories as the substrate envelope to avoid drug resistance coupled with the use of new scaffolds like the lysine sulfonamides [58,59] will result in novel inhibitors that avoid the need for boosting and reduce other long-term side effects.

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